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(54) Title: ORAL CYCLOSPORIN FORMULATIONS

(57) Abstract

Improved oral cyclosporin formulations which have high bioavailability and are capable of administration in hard capsules or nanoparticles are provided. In the subject formulations, cyclosporin is delivered in an orally acceptable vehicle comprising at least one alkanol solvent of from 2 to 3 carbon atoms in combination with at least one non-ionic surfactant. The subject formulations may further comprise at least one cosolvent, where cosolvents of interest include fatty acids and diols. The subject formulations find use in immunosuppressive therapy.

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ORAL CYCLOSPORIN FORMULATIONS

INTRODUCTION

Field of the Invention

The field of this invention is oral cyclosporin formulations.

Background

Despite efforts to avoid graft rejection through host-donor tissue type matching, in the majority of transplantation procedures where a donor organ is introduced into a host, immunosuppressive therapy is critical to the maintained viability of the donor organ in the host. A variety of immunosuppressive agents have been employed in transplantation procedures, including azathioprine, methotrexate, cyclophosphamide, FK-506, rapamycin and corticosteroids. Agents finding increased use in immunosuppressive therapy due to their preferential effect on T-cell mediated reactions are the cyclosporins.

Cyclosporins are a class of cyclic polypeptides consisting of eleven amino acids which are produced as a metabolite by the fungus species Tolypocladium inflatum Gams. Cyclosporins have been observed to reversibly inhibit immunocompetent lymphocytes, particularly T-lymphocytes, in the G_0 or G_1 phase of the cell cycle. Cyclosporins have also been observed to reversibly inhibit lymphokine production and release. Although a number of cyclosporins are known, Cyclosporin A is the most widely used.

Use of Cyclosporin A has been reported to prolong the survival of allogeneic transplants involving skin, heart, kidney, pancreas, bone marrow, small intestine and lung. In allogeneic transplantations, Cyclosporin A has been shown to suppress

humoral immunity and, to a greater extent, cell mediated immune reactions, including: allograft rejection, delayed hypersensitivity, experimental allergic encephalomyelitis, Freund's adjuvant arthritis, and graft vs. host disease. Although success has been realized with Cyclosporin A, following transplantation administration of the agent must be continued since the benefits of cyclosporin therapy are reversible and graft rejection occurs once administration of Cyclosporin A is discontinued.

Although cyclosporin formulations for both oral and intravenous administration have been developed, oral administration of cyclosporin is preferred because of the ease of administration and greater patient acceptance. Furthermore, intravenous administration of cyclosporin can result in anaphylactic reactions, a side effect not observed with oral formulations. Oral cyclosporin formulations which have been developed and are currently marketed include both soft gelatin capsule and solution formulations, both of which are sold under the trademarks SANDIMMUNE® and NEORAL™.

In using oral cyclosporin formulations in immunosuppressive therapy, both the care giver and manufacturer must be cognizant of many issues. With oral cyclosporin formulations, cyclosporin bioavailability can be limited because of cyclosporin's immiscibility in water and the tendency of cyclosporin to precipitate in aqueous environments. In addition, the concentration of cyclosporin present in oral formulations can be limited due to cyclosporin's hydrophobic nature. Furthermore, cyclosporin absorption by the gastrointestinal tract can be erratic from one formulation batch to the next, requiring constant monitoring of cyclosporin blood levels during treatment. Finally, packaging and storage stability are an issue with oral formulations. For example, with soft gelatin capsule formulations of cyclosporin, air tight packaging must be employed, which is inconvenient due to bulkiness and high cost. Furthermore, cyclosporine formulations may be unstable at lower temperatures, as cyclosporine crystallization may occur.

Thus, desirable oral cyclosporin formulations would be formulations that address at least some of the above issues. Ideally, oral formulations would promote high bioavailability, comprise high concentrations of cyclosporin and would be amenable to preparation in hard capsule form.

Relevant Literature

Physician's Desk Reference (1994) pp 2071-2074 describes oral cyclosporin formulations currently sold under the trademark SANDIMMUNE®.

Oral cyclosporine formulations are also described in the NEORAL™ package insert, (1995) (Sandoz Pharmaceuticals Corporation, East Hanover, New Jersey, 07936).

U.S. Patents of interest describing cyclosporins and derivatives thereof include: 4,220,641; 4,639,434; 4,289,851; and 4,384,996. U.S. Pat. No. 5,047,396 describes an intravenous preparation for administration of cyclosporin. U.S. Pat. Nos. 4,388,307; 4,970,076 and 4,990,337 describe the preparation of oral cyclosporin formulations.

The preparation of hard capsules for the oral delivery of pharmaceutical formulations is described in U.S. Pat. Nos. 4,822,618; 4,576,284; 5,120,710; and 4,894,235.

SUMMARY OF THE INVENTION

Oral cyclosporin formulations, and methods for their use in immunosuppressive therapy, are provided. In the subject formulations, cyclosporin is present in an orally acceptable vehicle comprising at least one alkanol solvent of from 2 to 3 carbon atoms in combination with at least one non-ionic surfactant. The subject formulations may further comprise one or more cosolvents, where cosolvents of interest are fatty acid esters and diols. The cyclosporin formulations can be packaged as hard capsules. By including a polyoxyalkylene surfactant, upon diluting of the stable dispersion into an aqueous medium, amorphous bioavailable cyclosporin nanoparticles are provided.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 provides the cyclosporin peak concentration (C_{max}) achieved in rats for several oral formulations according to the subject invention, where the C_{max} is shown as a relative value compared to the C_{max} achieved with SANDIMMUNE® ORAL formulation (SO).

Fig. 2 provides the time at which C_{max} occurred (T_{max}) for each of formulations shown in Fig. 1, where T_{max} is provided as relative value compared to the T_{max} of SANDIMMUNE® ORAL formulation (SO).

Fig. 3 provides the relative area under the blood concentration-time curve

(AUC) for each of the formulations shown in Fig. 1, where AUC is provided as a relative value compared to the AUC value for SANDIMMUNE® ORAL formulation (SO).

Fig. 4 provides the cyclosporin peak concentration (C_{max}) achieved in humans for several oral formulations according to the subject invention, as well as SANDIMMUNE® ORAL solution ("Sand" in the figure).

Fig. 5 provides the time at which C_{max} occurred (T_{max}) for each of formulations shown in Fig. 4.

Fig. 6 provides the area under the blood concentration-time curve (AUC) for each of the formulations shown in Fig. 4.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Oral cyclosporin formulations are provided which promote bioavailability and can be formulated as capsules, particularly hard capsules. In the subject formulations, cyclosporin is present in an orally acceptable vehicle comprising at least one alkanol solvent of from 2 to 3 carbon atoms in combination with at least one non-ionic surfactant. The subject formulations may further comprise at least one cosolvent, where cosolvents of interest include fatty acid esters and diols. Each of the components of the subject formulations are pharmaceutically acceptable. In addition to providing for high bioavailability, the subject formulations provide for reproducible cyclosporin absorption from one batch of a particular formulation to the next. The subject formulations find use in immunosuppressive therapy.

A number of cyclosporins are known in the art to exhibit immunosuppressive activity and may be delivered in the subject oral formulations. Cyclosporins that may be administered in the subject formulations include Cyclosporin A, Cyclosporin B, Cyclosporin C, Cyclosporin D and Cyclosporin G, as well as synthetic analogs thereof. *See* Merck Index (1989) 2759. The subject oral formulations are particularly suited for the delivery of Cyclosporin A. When delivered in the subject formulations, Cyclosporin A will be present in concentrations ranging from 50 to 150 mg/ml, usually 100 to 150 mg/ml, based on the volume of the vehicle component of the formulation.

The vehicle component of the subject formulations will include an alkanol

solvent component, where the alkanol solvent component will comprise at least one alkanol and usually no more than three different alkanols, more usually no more than two different alkanols, where the alkanols will usually be from 2 to 3 carbon atoms, and from 1 to 2 hydroxy groups, such that there is no more than 1 hydroxy group per 1.5 carbon atoms. Suitable alkanols include ethanol and propylene glycol. The total amount of alkanol solvent in the formulation will be at least about 1 % (v/v), usually at least about 3 % (v/v) and may be as high as 95 % (v/v), but will generally range from about 5 to 75 % (v/v), usually from about 5 to 60 % (v/v), and more usually from about 10 to 60% (v/v) of the formulation. When ethanol is present in the formulation as an alkanol solvent, the amount of ethanol may range from 5 to 20 % (v/v), usually from about 5 to 15 % (v/v) of the formulation, while when propylene glycol is present as an alkanol solvent, the amount of propylene glycol in the subject formulation may range from about 5 to 90 % (v/v), usually from about 5 to 85 % (v/v), more usually from about 10 to 50 % (v/v) of the formulation.

Also present in the orally acceptable vehicle will be at least one non-ionic polyoxyalkylene surfactant, usually not more than two polyoxyalkylene non-ionic surfactants. The polyoxyalkylene surfactants will have a hydrophilic-lipophilic-balance (HLB) of from about 5 to 20, usually from about 8 to 16. Preferably, the polyoxyalkylene non-ionic surfactants employed in the subject formulations will be polyoxyethylene compounds. Polyoxyethylene compounds of interest include: ethoxylated alcohols, *i.e.* polyoxyethylene alcohols or ethoxylated fatty alcohols, where the alcohol moieties are generally of from 10 to 18, usually from 10 to 14 carbon atoms, as well as ether and ester substituents thereof; and polyoxyethylene derivatives of fatty acid partial esters, usually monoesters, of polyols of from 4 to 6 carbon atoms, usually 6 carbon atoms, where the polyols may be polyol anhydrides *e.g.* sorbitan. The fatty acid moieties of the subject surfactant will typically range from 10 to 18 carbon atoms. The number of ethylenoxide groups will generally be in the range of 2 to 30, usually in the range from about 2 to 25. Preferred surfactants are polyoxyethylene (4) lauryl ether (BRIJ 30®) and polyoxyethylene (20) mono sorbitan mono-oleate (TWEEN 80®). The total amount of non-ionic surfactants present in the subject formulations will range from 5 to 65 %, usually from about 5 to 60 % (v/v) of the formulation. Where TWEEN 80® is present in the formulation, it will usually be present in amounts ranging

from 5 to 60 %, more usually from about 10 to 50 % (v/v) of the formulation. When BRIJ 30® is present in the subject formulation, it will usually be present in amounts ranging from 10 to 45 %, more usually from about 15 to 40 % (v/v) of the formulation.

The subject formulations may further comprise one or more cosolvents, usually not more than three different cosolvents, more usually not more than two different cosolvents, where suitable cosolvents include fatty acid esters and diols, where the cosolvent may be 100% fatty acid ester, 100% diol, or combination thereof. The total amount of cosolvent present in the formulation may range from about 20 to 80 % (v/v) and will usually range from about 25 to 75 % (v/v). When present in the formulation, the ratio of cosolvent to solvent in the subject formulations may range from about 1:1 to 15:1, but will usually range from about 1:1 to 13:1.

Fatty acid esters which may serve as cosolvents in the subject formulations are those fatty acid esters where the hydrocarbon chain of the fatty acid is from 12 to 18, usually 14 to 18 carbon atoms in length, where the fatty acid ester will be a mono-ester of a lower alkanol. Suitable fatty acid esters will generally comprise an even numbered fatty acid chain, where the hydrocarbon chain may be saturated or unsaturated, usually having not more than two sites of unsaturation. Fatty acids of interest will generally be of plant or mammalian origin and include palmitate, stearate, palmitoleate, linoleate, linolenate and the like, particularly myristate and oleate. The alcohol of the fatty acid mono-ester will be a lower alkanol of from 2 to 4 carbon atoms in length, usually 2 to 3 carbon atoms in length, with or without branches. Fatty acid esters of particular interest are isopropyl myristate and ethyl oleate. Isopropyl myristate, when present, will range from about 55 to 75 % (v/v), and ethyl oleate, when present, will range from about 35 to 75 % (v/v) of the total formulation. Usually the fatty acid ester will be present in an amount at least about equal (v/v) and up to 8 times the amount of surfactant in the formulation, usually not greater than 5 times the amount of surfactant in the formulation (v/v).

Diols may also be present in the subject formulations, where the diols may be present in addition to, or in lieu of, the fatty acid ester cosolvent. Diols of interest as cosolvents are generally liquids at physiologic temperatures and include diols of from 8 to 28 carbon atoms, usually 16 to 20 carbon atoms, where the diol may be a polyoxyalkylene diol, where alkylene is of from 2 to 3 carbon atoms. Suitable diols for

use as cosolvents may range from about 200 to 800 daltons, usually from about 200 to 650 daltons. Diols of particular interest include polyethylene glycols, particularly polyethylene glycol 200 (PEG_{200}), polyethylene glycol 400 (PEG_{400}), polyethylene glycol 600 (PEG_{600}), and the like, with PEG_{400} being preferred. When present as cosolvents in the subject formulations, the diols will range from about 5 to 60 % (v/v), usually from 5 to 55 % (v/v) of the formulation.

For formation of the amorphous nanoparticles, desirably in the formulation, the total amount of lower alkanol will generally be in the range of about 25-60 weight percent, more usually in the range of about 30-50 weight percent. The total amount of alkyleneoxy compound(s) will generally be in the range of about 20-50 weight percent, more usually in the range of about 25-40 weight percent. Where combinations of polyoxyalkylene compounds are employed, the amount of the fatty acid ester will generally range from about 25-100% of the polyoxyalkylene compounds.

In the subject formulations, the cosolvents themselves may impart desirable physical properties to the formulation, such as viscosity, stability and the like. Where desired, the formulation may further comprise additional agents which impart desired physical properties to the formulation, such as thickening agents, suspending agents, solidifying agents, and the like, where such agents include acacia, carboxymethyl-cellulose, hydroxypropylcellulose, lecithin, methyl cellulose, high molecular weight polyethylene glycols, e.g. those polyethylene glycols with molecular weights ranging from about 1000 to 6000, usually 1000 to 5000 daltons, povidone, sodium alginate, tragacanth, and the like . Also present in the subject formulations may be a number of minor components which provide various functions, such as enzyme inhibitors, preservatives, antioxidants, antimicrobial agents, stabilizers and the like. The total amount of these thickening agents and other additives, when present in the formulation, will normally not be greater than 5 weight %, usually 2 weight %, more usually 1 weight % of the formulation. A number of excipients may also be present in the subject formulations, as is known in the art.

The subject formulations are stable over a wide range of temperatures, where by stable is meant that the physical integrity of the formulation is not comprised, e.g. crystallization of the cyclosporin active agent does not occur. Included within the

temperature range over which the subject formulations are stable are lower temperatures, such as those employed in refrigerated storage, where such lower temperatures typically range from about 0 to 15°C, more typically from about 2 to 8 °C.

The subject formulations are suitable for administration in capsule form, *e.g.* hard and soft capsules. Methods of producing hard capsules comprising liquid formulations are known in the art and described in U.S. Pat. Nos. 4,822,618 and 4,576,284, the disclosures of which are herein incorporated by reference. Generally, hard capsules that find use with the subject formulations will comprise two parts: a shell component and a cap component. The shell and cap components fit together to produce an enclosed cavity of defined volume sealed in a hard capsule shell. The shell and cap components may be fabricated from a hydrophilic polymer, such as starch or gelatin. In preparing the hard capsules, the liquid formulation will be poured into the shell component and then the capsule will be sealed by fitting the cap component over the shell component. The seal between the two components may be secured, thereby preventing leakage of the enclosed formulation from the capsule, by using a sealant as described in EP 116744, the disclosure of which is herein incorporated by reference. To avoid degradation in the stomach, capsules comprising the subject formulations may be coated with an enteric coating which inhibits degradation of the capsule in the acidic environment of the stomach. A variety of enteric coatings are known in the art. *See for example*, U.S. Pat. No. 5,206, 219, the disclosure of which is herein incorporated by reference.

The compositions, particularly the nanoparticle producing formulation, may be prepared by first dissolving the cyclosporin in the lower alkanol, where a small proportion of the polyoxyalkylene compound may also be included, generally less than about 50 weight percent of the composition used for dissolving the cyclosporin. An elevated temperature may be employed, usually in the range of about 60 to 90°C. After dissolving the cyclosporin, the major proportion of the polyalkyleneoxy compound may be added and the total formulation brought to the desired ratios by the addition of the appropriate components. Generally, the cyclosporin can be dissolved in the lower alkanol (optionally including a portion of the polyalkyleneoxy compound) at a weight ratio of about 1:1.5-5, more usually

1:2-4.

The subject formulations find use in immunosuppressive therapy. Immunosuppressive therapy is indicated in a wide variety of diseases, including idiopathic nephrotic syndrome, type I insulin-dependent diabetes, Behçet's syndrome, active Crohn's disease, aplastic anemia, severe corticosteroid-dependent asthma, psoriasis, rheumatoid arthritis, and other diseases where the immune system may play a pathogenic role. Of particular interest is the use of the subject formulations in transplant situations, including both allogeneic and xenogeneic organ, tissue or cell transplantation, where immunosuppression is desired to ensure maintained viability of the transplanted organ or tissue or cell following transplantation, *i.e.* to prevent graft rejection or prevent graft vs. host disease, *e.g.* following bone marrow transplantation.

In using the subject formulations to provide immunosuppressive therapy to a host, an effective amount of cyclosporin will be orally administered to achieve the desired level of immunosuppression in the host, depending on the particular condition to be treated. With transplantation, usually an initial dosage of cyclosporin will be administered prior to operation. Following transplantation of the donor organ to the host, the cyclosporin will be administered repeatedly, *i.e.* chronically, to the host to maintain immunosuppression. The initial dosage will be administered 4 to 12 hours prior to transplantation and may range from 10 to 18 mg/kg host, usually 10 to 15 mg/kg host. Following the operation, the initial dosage will usually be continued on a daily basis for a period of 1 to 3 weeks, usually 1 to 2 weeks. The dosage may then be tapered to a maintenance dosage of 3 to 10 mg/kg per day, usually 3 to 6 mg/kg per day. The rate at which the dosage is tapered to the maintenance level may range from 3 to 8 % per week and will usually be about 5 % per week. The dosage will typically be adjusted based on trough blood levels to maintain a concentration of 150 to 250 ng/ml, as measured by HPLC, RIA, ELISA or TDx assay. The subject formulations may be administered in conjunction with additional agents, where adjunct therapy is recommended and is known in the art. For example, the subject formulations may be administered in conjunction with adrenal corticosteroids, azathioprine and the like.

Administration of the subject formulations in conjunction with transplantation of a donor organ to a host will result in a prolongation of the viability of the donor organ in the host as a result of suppression of the host's immune response to the

presence of the donor organ. By "prolongation of viability" is meant that the donor organ remains viable in the host for a longer period of time than it would have had immunosuppressive therapy not been employed in conjunction with the transplantation. Thus, prolongation of viability includes maintenance of viability for an indefinite period of time. A donor organ is considered viable as long as it maintains functionality in the host environment.

For convenience of the user, kits may be provided having the appropriate amount of cyclosporin, one or more dosage levels and the cosolvents, namely the lower alkanol(s) and the polyalkyleneoxy compound(s), e.g. at least one of ethanol and propylene glycol, and at least one of polysorbate 80 and PEG400.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Several oral cyclosporin formulations according to the subject invention were prepared. The bioavailability of cyclosporin in the prepared formulations was then observed in rats and humans.

I. *Oral Cyclosporin Formulations*

The following oral Cyclosporin A formulations were prepared. In each case, 100 mg CsA, the indicated amount of surfactant, and the indicated amount of ethanol or propylene glycol were added to a 1.0 ml volumetric flask, and the final volume of 1.0 ml was achieved by addition of a suitable volume of fatty acid ester and/or diol.

Formulation	Composition		
19	EtOH Tween 80 IM	0.1 ml 300 mg q.s. to 1.0 ml	(10%) (0.278 ml) ⟨(0.622 ml) (531 mg)
20	EtOH Brij 30 IM	0.05 ml 350 mg q.s. to 1.0 ml	(5%) (0.368 ml) ⟨(0.582 ml)(496 mg)
21	PG Brij 30 IM	0.05 ml 350 mg q.s. to 1.0 ml	(5%) (0.368 ml) ⟨(0.582 ml)(496 mg)

Formulation	Composition		
22	EtOH Tween 80 EO	0.1 ml 300 mg q.s. to 1.0 ml	(10%) (0.278 ml) ⟨(0.622 ml)(541 mg)
24	EtOH Brij 30 EO	0.05 ml 350 mg q.s. to 1.0 ml	(5%) (0.368 ml) ⟨(0.582 ml) (506mg)
24	PG Brij 30 EO	0.05 ml 350 mg q.s. to 1.0 ml	(5%) (0.368 ml) ⟨(0.582 ml) (506mg)
33	EtOH Brij 30 IM	0.1 ml 150 mg q.s. to 1.0 ml	(10%) (0.158 ml) ⟨(0.742 ml)(633 mg)
34	EtOH Brij 30 EO	0.1 ml 150 mg q.s. to 1.0 ml	(10%) (0.158 ml) ⟨(0.742 ml)(646 mg)
35	EtOH Tween 80 PG	0.1 ml 500 mg q.s. to 1.0 ml	(10%) (0.463 ml) ⟨(0.437 ml)(453 mg)
36	EtOH Tween 80 PG EO	0.1 ml 300 mg 100 mg q.s. to 1.0 ml	(10 %) (0.278 ml) (0.097 ml) ⟨(0.525 ml)(465 mg)
37	EtOH Tween 80 PEG 400 EO	0.1 ml 300 mg 100 mg q.s. to 1.0 ml	(10 %) (0.278 ml) (0.088 ml) ⟨(0.534 ml)(464 mg)
38	EtOH Brij 30 PG EO	0.1 ml 300 mg 100 mg q.s. to 1.0 ml	(10 %) (0.316 ml) (0.097 ml) ⟨(0.487 ml)(424 mg)
39	EtOH Brij 30 PG EO	0.1 ml 300 mg 200 mg q.s. to 1.0 ml	(10 %) (0.316 ml) (0.193 ml) ⟨(0.391ml)(340 mg)
40	PG Brij 30 EO	300 mg 300 mg q.s. to 1.0 ml	(290 ml) (0.316 ml) ⟨(0.394 ml)(343 mg)
41	EtOH Brij 30 Tween 80 EO	0.05 ml 150 mg 100 mg q.s. to 1.0. ml	(5%) (0.158 ml) (0.093 ml) ⟨(0.649 ml) (565 mg)
42	PG Brij 30 Tween 80 EO	0.05 ml 150 mg 100 mg q.s. to 1.0. ml	(5%) (0.158 ml) (0.093 ml) ⟨(0.649 ml) (565 mg)

Formulation	Composition		
43	EtOH Tween 80 PG	0.10 ml 400 mg q.s. to 1.0 ml	(10 %) (0.371 ml) (0.529 ml)
44	EtOH Tween 80 PEG_{400}	0.10 ml 400 mg q.s. to 100 ml	(10%) (0.371 ml) ((0.529 ml)(601 mg)
45	EtOH Tween 80 PG PEG_{400}	0.10 ml 300 mg approx.250mg approx.250mg	 (0.278 ml) (0.243 ml) (0.220 ml)
46	EtOH Tween 80 PG	0.10 ml 100 mg q.s. to 1.0 ml	(10%) (0.093 ml) (0.807 ml)
48	EtOH Tween 80 PG PEG_{400}	0.10 ml 200 mg approx.250mg approx.250mg	 (0.186 ml) (0.243 ml) (0.220 ml)
49	EtOH Tween 80 PG	0.10 ml 600 mg q.s. to 1 ml	(10 %) (0.558 ml) (0.342 ml)
50	EtOH Tween 80 PG	0.10 ml 300 mg q.s. to 1.0 ml	(10%) (0.278 ml) (0.622 ml)
51	EtOH Tween 80 PG	0.10 ml 200 mg q.s. to 1.0 ml	(10 %) (0.186 ml) (0.714 ml)
52	EtOH Tween 80 PG	0.05 ml 400 mg q.s. to 1.0 ml	(5%) (0.371 ml) (0.579 ml)

PG = Propylene Glycol; EtOH = ethanol
Brij 30 = polyoxyethylene (4) lauryl ether
Tween 80 = polyoxyethylene (20) mono sorbitan mono-oleate
IO=isopropyl myristate
EO=ethyl oleate

II. *In vivo Bioavailability Studies for Formulations 19-24 and 33-42*

The bioavailability of cyclosporin in formulations 19-24 and 33-42 was studied as follows. As a measure of bioavailability, the following pharmacokinetic parameters were determined: (a) the peak blood concentration of cyclosporin (C_{max}); (b) time required to attain C_{max} (T_{max}); and the area under the blood concentration time-curve time (AUC). In addition to formulations 19-24 and 33-42, the bioavailability of

cyclosporin in SANDIMMUNE® Oral Solution (SO) under analogous conditions was observed for comparison purposes. For each of the above formulations, CsA-naive Sprague Dawley rats weighing 250-350 gm were fed pelletized standard food (Agway® 3000, Granville Mill, Greensboro, NC) and water *ad libitum*. One day prior to the experiment, silicone rubber cannulae were inserted into the right jugular and right femoral veins under light ether anesthesia. After overnight fast, CsA was administered by gavage.

Following administration, 200µl blood samples were collected from the jugular vein in 0.5 ml polypropylene microfuge tubes containing 0.3 mg of lyophilized Na EDTA and vortexed immediately for 10 sec. The sampling times for animals subjected to oral formulations were 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48 and 72 hr after administration.

CsA, including some of its metabolites, was determined in whole blood by fluorescent polarization immunoassay (FPI)(TDx, Abbot Lab.). Briefly, 150 µl of the whole blood sample were quantitatively transferred to a 1.5 ml microfuge tube. Cells were lysed and dissolved with 50 µl of a surfactant-containing solubilizing reagent. Proteins were then precipitated out with 300 µl of acetonitrile. After centrifugation, the supernatant was subjected to the FPI assay in a TDx Autoanalyzer following the procedure recommended by Abbott Diagnostics. Since the TDx assay was originally developed for human blood, some of the recommended procedures were modified as follows. A series of standard solutions of known CsA concentration were prepared by adding a known amount of CsA to rat blood treated with EDTA. When the CsA concentration in a sample was expected to be greater than 1.0 µg/ml, the blood sample was diluted 10-fold in a 0.1 M-phosphate buffer at pH 7.0. For diluted samples, another calibration curve was made using a series of standard solutions containing known amounts of CsA, which is volume-wise 10% in rat blood and 90% phosphate buffer.

Descriptive pharmacokinetic parameters were obtained from non-compartmental analyses. The peak concentration (C_{max}) and the time at which the peak concentration occurred (T_{max}) were estimated by inspection of the raw concentration-time profile for each rat. The area under the blood concentration-time curve (AUC) from time 0 through the last data point (AUC_{0-t}) was calculated according to the linear

trapezoidal procedure. The residual area under the tail of the blood concentration-time curve ($AUC_{t-\infty}$) was estimated as the ratio of the final observed concentration (C*) to the first-order rate constant associated with the terminal elimination phase of the concentration-time profile (λ_z). The rate contact λ_z was determined by log-linear regression of the concentration-time data in the apparent terminal log-linear phase of the concentration-time profile (i.e., the final 3 to 5 data points, depending on the profile under analysis). The total AUC ($AUC_{t-\infty}$) was taken as the sum of AUC_{0-t} and $AUC_{t-\infty}$.

The results for each formulation were compared with the results obtained for SO, and are provided in Figs. 1-3. The results demonstrate that, for the majority of the formulations, greater bioavailability of cyclosporin is achieved with the subject formulations as compared with SANDIMMUNE® Oral Solution (SO), as indicated by the higher AUC values of the subject formulations.

III. *In vivo Human Bioavailability of Formulations 35, 43-46 and 48-52.*

48 healthy males between the ages of 19 and 55 with no more than 20 % deviation from ideal weight were used as test subjects. A single dose, fasted, randomized, double-blinded, three-way crossover study was conducted. The 48 subjects were randomized into 6 groups of 8 subjects. Each group received a single 300 mg dose of cyclosporin from the above formulations, or SANDIMMUNE® Oral Solution (SO), on three different occasions, where each occasion was separated by a 7-day washout period.

Subjects were required to fast 10 hours prior to, and 4 hours after, dosing. Water was allowed ad lib during the study, except for a 1 hour period prior through 2 hours following dosing. Prior to dosing, a 15 ml blood sample was drawn. For administrations, 3 ml aliquots of formulation (300 mg) was combined with 200 ml chocolate milk and orally ingested. 10 ml blood samples were drawn at t= 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours. A post study 15 ml blood sample was also drawn.

Concentrations of cyclosporin A in the whole blood samples were assayed using the TDx (Abbott Diagnostics, N. Chicago, IL) according to the manufacturer's instructions.

Non-compartmental pharmacokinetics were derived using standard methods. The maximum whole blood concentration (C_{max}) and the time of its occurrence (T_{max}) were compiled from the concentration-time data. The area under the blood concentration time curve (AUC) was calculated by the linear trapezoidal rule to the last blood concentration above the limit of sensitivity (25 ng/ml) and extrapolated to infinity.

The observed C_{max}, T_{max} and AUC values for each formulation were averaged. The average values for each formulation are provided in Figures 4-6. The results demonstrate that for each formulation tested, Cmax occurred at least twice as fast as with SANDIMMUNE® Oral Solution (SO) under the same conditions. Furthermore, the AUC observed for the test formulations was at least 2000 ng•hr/ml greater than that observed for SANDIMMUNE® Oral Solution (SO) under the same conditions. Based on these results, formulations 35, 43-46 and 48-52 provide for greater bioavailability than SANDIMMUNE® Oral Solution (SO).

Formulations were prepared for the formationof amorphous nanoparticles on dilution in an aqueous medium.

IV. *Nanoparticle Formulations*

A. 5 g of cyclosporin A was added to 5 mL of ethanol. The mixture was stirred to complete dissolution of cyclosporin A. To the resulting solution were added 25 g of polysorbate 80 and the volume is completed to 50 mL by 1,2-propylene glycol. The mixture was sufficiently stirred at room temperature until a homogeneous solution was formed.

B. 5 g of cyclosporin A was added to 5 mL of ethanol. The mixture was stirred until complete dissolution of cyclosporin A. To the resulting solution were added 15 g of polysorbate 80 and the volume is completed to 50 mL by a mixture of 1,2-propylene glycol and polyethylene glycol 400. The mixture was sufficiently stirred at room temperature until a homogeneous solution was formed.

C. 1 mL of the solution obtained in example 1 was added in 50 mL of water with a glass syringe as recommended for the oral administration of

concentrated emulsions or microemulsions in human. The addition of the solution was followed by a quick dissolution and a white suspension of fine particles was obtained having a blue reflect as colloidal suspensions (Tyndall effect). After centrifugation at 26,000 g during 5 hours, the sediment was washed with water and then centrifuged at 26,000 g during 24 hours. The washing and centrifugation processes were repeated twice under the same conditions. After drying, an x-ray powder diagram was performed. The solid was exclusively in amorphous form.

The sediment was examined by scanning electron microscopy. The sediment was constituted of amorphous spheric nanoparticles with a diameter between 200 and 400 nm with the presence of some aggregates.

D. 2 mL of the solution obtained in example 1 was added in 100 mL of water and the colloidal suspension was examined 10 minutes and 1 hour after the dilution by a diffraction/diffusion laser granulometer (Malvern SB.OD).

After 1 hour, two particle populations were observed: one representing 70% of the weight of cyclosporin A with an average diameter of 300 nm and a second one representing 30% of the weight of cyclosporin A with an average diameter of 20 μm, probably constituting aggregates of nanoparticles.

E. 1 mL of the solution obtained in example 1 was added to 50 mL of water and the colloidal suspension was stirred during 10 minutes.

The suspension was then added to 200 mL of artificial acidic gastric juice and warmed at 37°C. The homogeneous colloidal suspension was examined by diffraction/diffusion laser granulometry (Malvern SB.OD). The suspension was constituted exclusively of nanoparticles with an average diameter of 600 nm.

F. 1 mL of the solution obtained in example 1 was added directly to 200 mL of artificial acidic gastric juice.

The homogeneous suspension was warmed at 37°C and examined rapidly by diffraction/diffusion laser granulometry (Malvern SB.OD). The suspension was exclusively constituted of nanoparticles with an average diameter of 350 nm.

From the above results and discussion, it is evident that novel cyclosporin formulations having high bioavailability are provided. The subject formulations are

capable of comprising high concentrations of cyclosporin and are storage stable over a wide range of temperatures, including low temperatures commonly used in refrigeration. The subject formulations are amenable to delivery in capsule form, including hard capsule form, providing for ease of storage and handling. The formulations also provide amorphous nanoparticles, which result in enhancced bioavailability of the cyclosporin.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A cyclosporin formulation consisting of:

cyclosporin;

at least one alkanol solvent of from 2 to 3 carbon atoms; and

at least one non-ionic polyoxyalkylene surfactant, wherein said surfactant is selected from the group consisting of polyoxyethylene alcohols and fatty acid monoesters of ethoxylated polyols of from 4 to 6 carbon atoms.

2. The formulation according to Claim 1, wherein said formulation further consists of at least one cosolvent selected from the group consisting of mono-esters of a lower alkanol and a fatty acid of from 14 to 18 carbon atoms and diols of from 8 to 28 carbon atoms.

3. The formulation according to Claim 1, wherein said alkanol solvent is from about 5 to 75 % (v/v) of said formulation, said at least one non-ionic polyoxyalkylene surfactant is from about 5 to 65 % (v/v) of said formulation, and said at least one cosolvent is from about 20 to 80 % (v/v) of said formulation.

4. A cyclosporin formulation consisting of:

Cyclosporin A;

at least one alkanol solvent selected from the group consisting of ethanol and propylene glycol, wherein said alkanol solvent is from about 5 to 75 % (v/v) of said formulation;

at least one non-ionic polyoxyethylene surfactant, wherein said non-ionic polyoxyethylene surfactant is selected from the group consisting of polyoxyethylene alcohols and mono-esters of ethoxylated sorbitans, and is from about 5 to 65 % (v/v) of said formulation; and

at least one cosolvent, wherein at least one of said cosolvents is an ester of a lower alkanol of from 2 to 4 carbon atoms and a fatty acid of from 14 to 18 carbon atoms, wherein said cosolvent is from about 20 to 80 % (v/v) of said formulation.

5. The formulation according to Claim 4, wherein said non-ionic surfactant is selected from the group consisting of polyoxyethylene (4) lauryl ether and polyoxyethylene (20) mono sorbitan mono-oleate, said fatty acid ester is selected from the group consisting of isopropyl myristate and ethyl oleate, and said formulation comprises two cosolvents, wherein one of said cosolvents is a diol of from 8 to 28 carbon atoms.

6. An oral cyclosporin formulation consisting of:

Cyclosporin A at a concentration ranging from about 50 to 150 mg/ml;

an alkanol solvent component consisting of ethanol and propylene glycol, wherein said alkanol solvent component is from about 5 to 75 % (v/v) of said formulation; and

a mono-ester of an ethoxylated sorbitan in from about 10 to 50 % (v/v) of said formulation.

7. The formulation according to Claim 6, wherein said ethanol is from about 5 to 20 % (v/v) of said formulation.

8. The formulation according to Claim 6, wherein said propylene glycol is from about 10 to 50 % (v/v) of said formulation.

9. A hard capsule cyclosporin formulation consisting of:

a hard capsule containing the oral formulation according to Claim 1.

10. An aqueous dispersion of cyclosporin nanoparticles, wherein at least 50 weight percent of the cyclosporin present in the dispersion is of particles less than about 1 μm, said cyclosporin being amorphous.

11. A dispersion according to Claim 10, comprising in minor amounts lower alkanol and at least one polyoxyethylene surfactant.

12. A dispersion according to Claim 11, wherein said lower alkanol is at least

one of ethanol and propylene glycol and said polyoxyethylene compound is polysorbate 80.

13. A kit comprising cyclosporin, at least one of ethanol and propylene glycol, and at least one of polysorbate 80 and PEG 400.

14. A method for preparing an aqueous dispersion of cyclosporin particles according to Claim 10, said method comprising:

combining at least one of ethanol and propylene glycol with cyclosporin to form a solution; and

combining said solution with a polyethyleneoxy surfactant to form a second solution, which upon dilution with water forms amorphous nanoparticles of said cyclosporin.

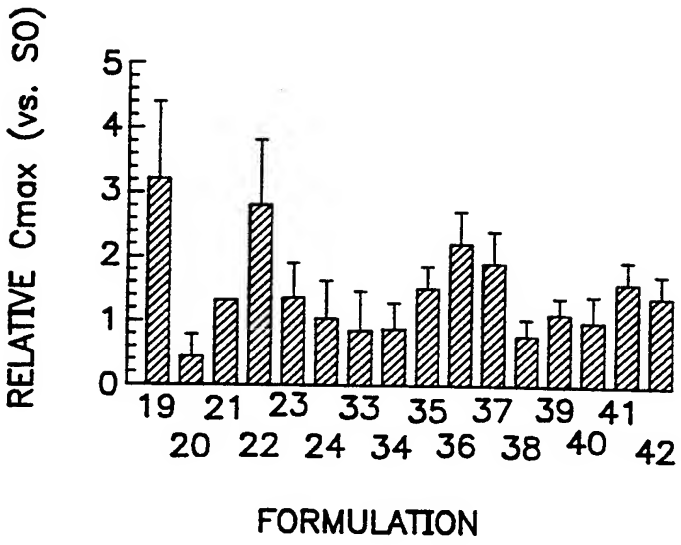
RELATIVE Cmax (vs. SO)
0
1
2
3
4
5
19 21 23 33 35 37 39 41
20 22 24 34 36 38 40 42
FORMULATION

FIG. 1

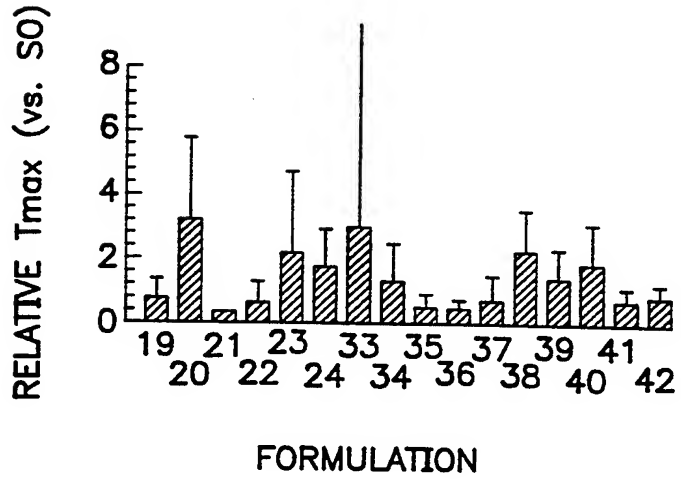
RELATIVE Tmax (vs. SO)
0
2
4
6
8
19 21 23 33 35 37 39 41
20 22 24 34 36 38 40 42
FORMULATION

FIG. 2

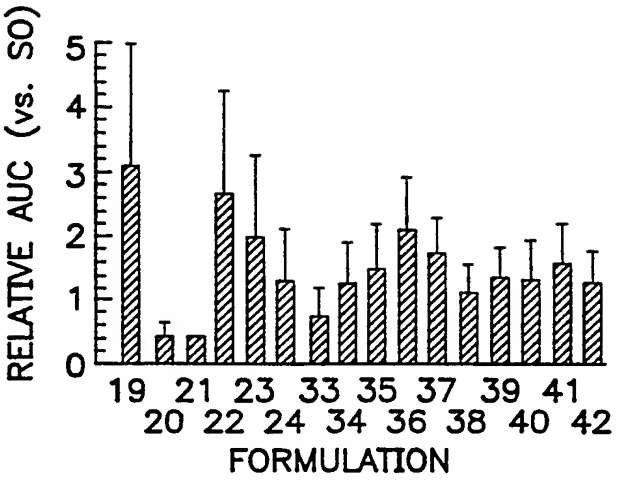
RELATIVE AUC (vs. SO)
0
1
2
3
4
5
19 21 23 33 35 37 39 41
20 22 24 34 36 38 40 42
FORMULATION

FIG. 3

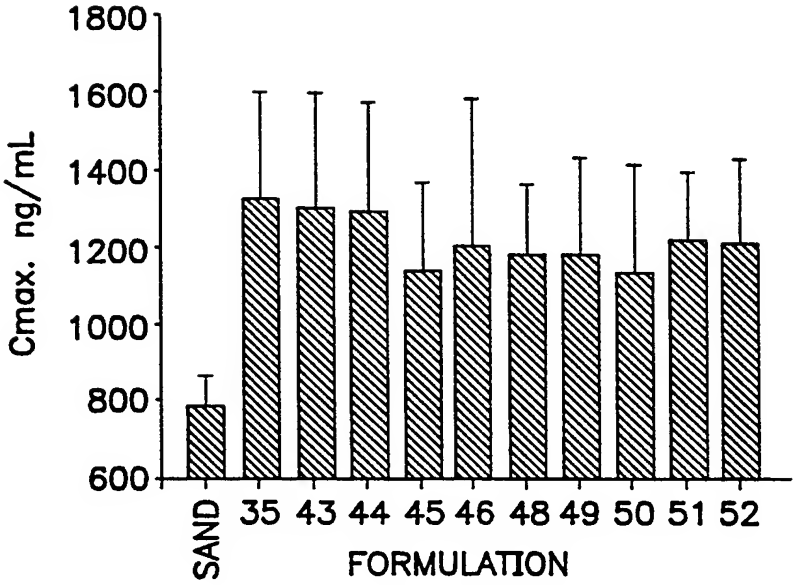
Cmax. ng/mL
600
800
1000
1200
1400
1600
1800
SAND 35 43 44 45 46 48 49 50 51 52
FORMULATION

FIG. 4

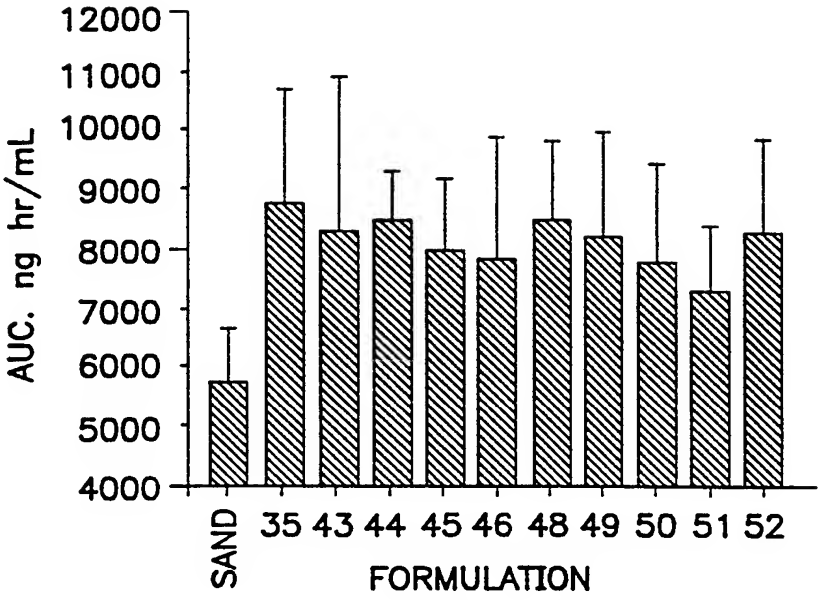
12000
11000
10000
9000
8000
7000
6000
5000
4000
AUC. ng hr/mL
SAND
35 43 44 45 46 48 49 50 51 52
FORMULATION

FIG. 5

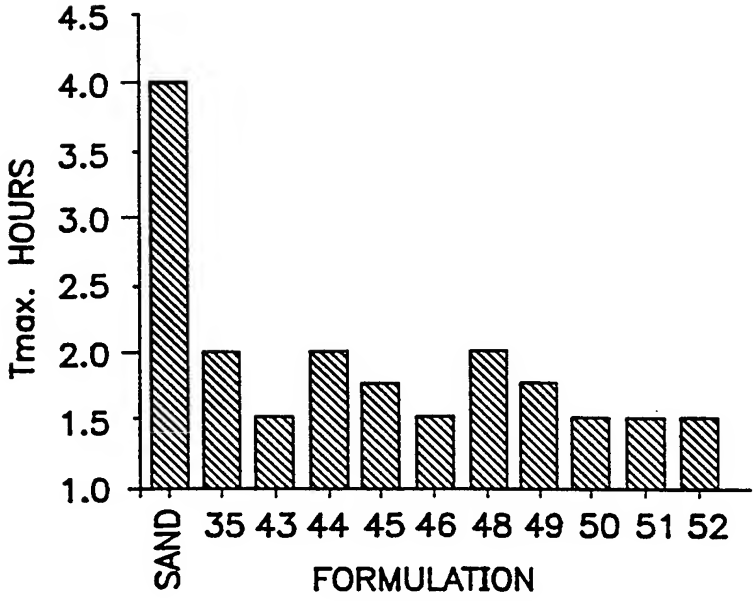
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Tmax. HOURS
SAND
35 43 44 45 46 48 49 50 51 52
FORMULATION

FIG. 6

INTERNATIONAL SEARCH REPORT

Int.. ..ational application No.
PCT/US96/12569

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/48, 47/34
US CL :424/456; 514/885, 962, 975

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/456; 514/885, 962, 975

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2,106,827 A1 (F. RICHTER) 26 March 1994, page 1, lines 4-6, page 2, lines 10-14, page 3, lines 7-9,16-30, page 4 lines 18-20, page 8 , lines 13-15, and page 9, lines 15-17.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 17 OCTOBER 1996	Date of mailing of the international search report 29 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer EDWARD J. WEBMAN Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*